

The University of Chicago

HISTOLOGICAL AND CYTOLOGICAL
RESPONSES OF ROOTS TO
GROWTH-REGULATING
SUBSTANCES

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY
SEPTEMBER, 1943

By
WILLIAM MARION CARLTON

Reprinted from
THE BOTANICAL GAZETTE, Vol. 105, No. 2, December, 1943

The University of Chicago

HISTOLOGICAL AND CYTOLOGICAL
RESPONSES OF ROOTS TO
GROWTH-REGULATING
SUBSTANCES

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BOTANY
SEPTEMBER, 1943

By
WILLIAM MARION CARLTON



Reprinted from
THE BOTANICAL GAZETTE, Vol. 105, No. 2, December, 1943

HISTOLOGICAL AND CYTOLOGICAL RESPONSES OF ROOTS TO GROWTH-REGULATING SUBSTANCES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 557

WILLIAM M. CARLTON

Introduction

Gross responses of plant organs to growth-regulating substances have been studied widely during the past decade. Histological and cytological studies of the effects of such substances on stems and other aerial organs have been reported by many investigators (1, 2, 4, 9, 11, 12, 13, 14, 15). Studies of the effects of such substances on plant roots, however, have been limited largely to gross observations on the stimulation or inhibition of root elongation, the development of laterals, and the physiological activity of the roots. Only recently have the histological and cytological changes induced been considered.

According to THIMANN (20), NIELSON first demonstrated (in 1930) the inhibition of root elongation by the external application of auxins, using in his treatment a crude extract from *Rhizopus* cultures. Later (in 1934) KÖGL, HAAGEN-SMIT, and ERXLEBEN found that pure auxin a, auxin b, and indole(3)acetic acid had a similar effect. THIMANN found that indene(3)acetic acid applied externally also inhibited elongation, and he advanced the suggestion that perhaps this inhibition is a general property of growth-regulating substances.

ZIMMERMAN and HITCHCOCK (21) found that the normally unbranching aerial roots of grape could be induced to form numerous lateral roots and that elongation was retarded by treatment of the region with any of several chemicals in lanolin paste or by immersing the root tips in aqueous solutions of the substances.

LANE (16) found that indole(3)acetic

acid is a specific inhibitor of root growth in young *Avena* seedlings and that the inhibition is accompanied by some thickening of the root. Stunting effects on roots also were reported by THIMANN, MARMER (19), MACHT and GRUMBEIN (18), and BONNER and KOEPFLI (5). These effects were studied chiefly on roots of cereal grains and legumes. BORGSTROM (6) investigated the effects of a number of growth substances on bulb-forming plants (*Allium cepa*, *A. fistulosum*, and *Crocus*), determining effective concentrations, toxicity limits, and gross morphological effects. He found that elongation of roots was inhibited by treatment with all but the most dilute concentrations, and inhibited elongation often was accompanied by enlargement of the root tips.

The effectiveness of growth-regulating substances in the induction of lateral root formation has been observed in various plants (21, 20, 16, 19, 8, 6). MARMER (19) reported excessive root-hair formation following treatment of wheat seedlings with indoleacetic, indolebutyric, and indolepropionic acids at pH 4.6 but not at pH 7.5.

The histological and cytological responses of roots of species of *Allium* to several chemicals has been investigated by LEVAN (17). BURSTRÖM (7) has discussed the morphological changes induced in roots of wheat following treatment with heteroauxin.

The present investigation was undertaken for the purpose of extending information on the histological and cytological changes which occur in roots following treatment with various growth-regulating substances.

Material and methods

The roots used were those of bulb-forming monocotyledons. The species selected were *Allium cepa*, *Narcissus* (var. Paper White), and *Tulipa* (vars. John Ruskin and Louis XIV), but not all were treated with all of the chemicals used.

Six growth-regulating substances were used: α -naphthaleneacetic acid, indole-(3)acetic acid, indole(3)butyric acid, β -naphthoxyacetic acid, α -naphthyl acetamide, and tryptophane. They were used in concentrations of 0.001 or 0.002%, 10 or 20 p.p.m., dissolved in a three-salt nutrient solution. Because of the difficulty in getting some of the substances into solution in an aqueous medium, all but tryptophane and naphthyl acetamide were first dissolved in small amounts of ethyl alcohol, in a manner similar to that employed by MACHT and GRUMBEIN (18); later, measured amounts of the alcoholic solutions were pipetted (with constant agitation) into definite volumes of nutrient solution to give the desired concentrations. The naphthyl acetamide, being relatively insoluble in ethyl alcohol, was put into solution in hot *n*-butyl alcohol. Tryptophane was dissolved directly in the measured volume of nutrient solution.

The bulbs were rooted by suspending them on wire-gauze racks over containers of nutrient solution so that the basal portions were submerged. When the roots had reached a satisfactory length, 1 inch or more in case of *Allium* and *Narcissus* and somewhat less in the case of *Tulipa*, individual bulbs were removed from the racks, rinsed with water, and suspended in tumblers so that the roots were completely immersed in nutrient solution to which the respective substances had been added.

Length of treatment varied from 24 to 72 hours, being generally 48 hours. At the conclusion of the treatment the bulbs were removed from the growth-substance solution, rinsed in distilled water, and transferred to tumblers of regular nutrient solution. Roots were cut at even intervals from 24 to 216 hours after the bulbs were replaced in nutrient solution, and fixed in Navashin's solution under reduced pressure.

The fixed roots were dehydrated by the tertiary butyl-alcohol method, imbedded in paraffin, sectioned, and stained in a modification of Flemming's triple stain. All sections were cut at 15 μ , with the exception of the longitudinal sections of the *Tulipa* roots. These, because of their small size, were sectioned at 10 μ .

Observations

GROSS RESPONSES

No definite responses of the roots were noticed for approximately 24 hours after replacing the bulbs in nutrient solution following treatment with the growth substance solutions; but between 24 and 72 hours after their return, several changes were noted.

All the chemicals in the concentrations used resulted in inhibition of root elongation, either temporary or permanent. Following treatment with indoleacetic acid the roots soon began to elongate, and in some cases the root tips grew as much as 2 inches during the subsequent 3 weeks. The roots treated with the other chemicals showed no later elongation.

Often a swelling was observed near the root tip; the time of occurrence and the amount varied in the different species. *Allium* showed the quickest response, but *Narcissus* reacted almost as rapidly. The amount of terminal enlargement in

the roots of *Tulipa* was so slight as to be hardly discernible, and histological examination of longitudinal sections were required to determine it.

The terminal swellings began about 24 hours after replacement of the bulbs in nutrient solution. Their size increased and reached a maximum in 1-3 days.

The enlargements which developed on roots of *Allium* and *Narcissus* as a result of treatment with the indoleacetic, indolebutyric, naphthaleneacetic, and naphthoxyacetic acids were similar in appearance and somewhat larger in diameter than were those induced by tryptophane. They were frequently twice the diameter of the root and sometimes scarcely longer than thick, while those induced by tryptophane were slender and often $\frac{1}{2}$ inch or more in length.

About 200 hours after treatment, the enlargements at the tips of *Narcissus* roots treated with naphthaleneacetic began to produce root hairs. At 264 hours the enlargements were almost completely covered with them, none more than a small fraction of a millimeter in length. Root hairs were not induced by the other chemicals, nor were they formed on roots of the other species.

Root primordia and secondary roots developed only on *Allium*. This genus normally forms secondary roots, but there were more primordia, and these were closer to the terminal meristem than in untreated bulbs. The secondary roots were much stunted and enlarged, and some of them had primordia (fig. 4).

HISTOLOGICAL AND CYTOLOGICAL RESPONSES

In *Allium* the characteristic enlargement of the root tip following treatment reaches maximum size within 48 hours. Transition from normal tip to swollen area is rather gradual and covers about

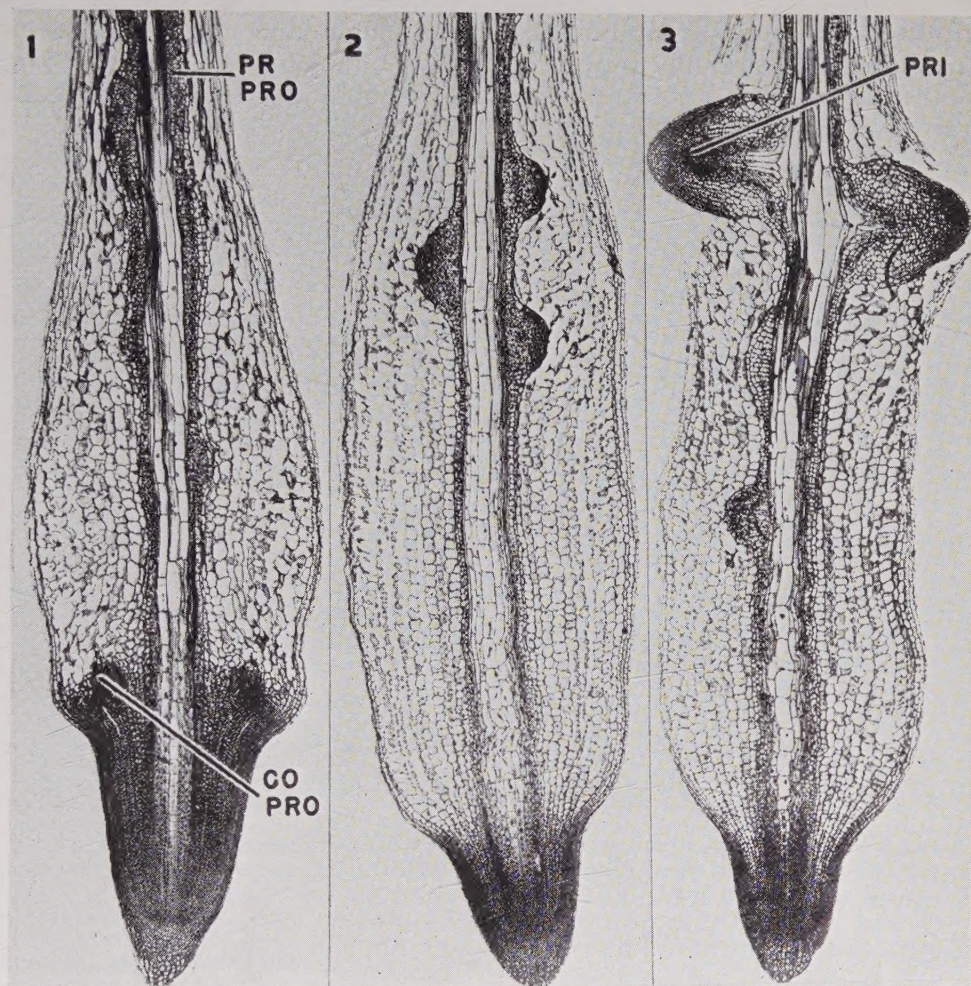
20 cell lengths. Within 48 hours the pericycle cells for some distance back from the apex of the root undergo transverse divisions which result in the formation of longitudinal rows of short, meristematic cells, which divide in several planes (figs. 1, 2). Tangential division of the cells of the pericycle becomes prominent in 72 hours, and within 96 hours definite root primordia are developed. About 264 hours are required for the primordia to grow through to the epidermis (fig. 8). Within 360 hours many have begun to emerge (figs. 3, 5, 7). Some are much enlarged and in turn have formed primordia (fig. 4).

While cell proliferation is much more frequent in the pericycle than in any other tissue of the root, it may also occur in other tissues, such as the cortex (figs. 1, 6). Proliferation, when it occurred, was in the form of a band centrifugal to the stele and extending almost to the epidermis (figs. 1, 6). Such divisions in the cortex of the embryonic tip occur early in ontogeny. They are at first radial or diagonal, but in older material many divide tangentially. Division is not pronounced in the larger cells of the swollen region. Some division also occurs in the mature cells far above the terminal enlargement.

Roots of John Ruskin and Louis XIV *Tulipa* do not develop noticeable swollen tips after treatment. The earliest response is the altered cell polarity in the immature cortex. As early as 48 hours after treatment, and in all cases thereafter, these cells show evidence of recently completed divisions in all planes. Close to the tip the divisions are predominantly radial or diagonal, but at higher levels many of them are tangential (figs. 9, 10). For the first 48 hours after treatment the divisions are limited almost exclusively to the meristematic re-

gion, but after 72 hours divisions in several planes occur in mature cells (fig. 10).

formed remaining slender and undergoing no enlargement. Subsequently the pericyclic cells divide further and form



FIGS. 1-3.—Longisections of *Allium* root tips treated with 0.001% naphthaleneacetic acid: Fig. 1, 120 hours after treatment, showing proliferation of pericycle and cortex. Fig. 2, 216 hours, showing root primordia. Fig. 3, 360 hours, showing primordia emerging. Figs. 2 and 3 show transition from normal to enlarged regions. *Pr pro*, proliferation of pericycle; *Co pro*, proliferation of cortex; *Pri*, root primordium.

Transverse divisions are initiated in the pericycle approximately 72 hours after treatment and continue for 72 hours or more. These divisions are confined to limited areas. Tangential divisions first occur about 168 hours after treatment, the daughter cells thus

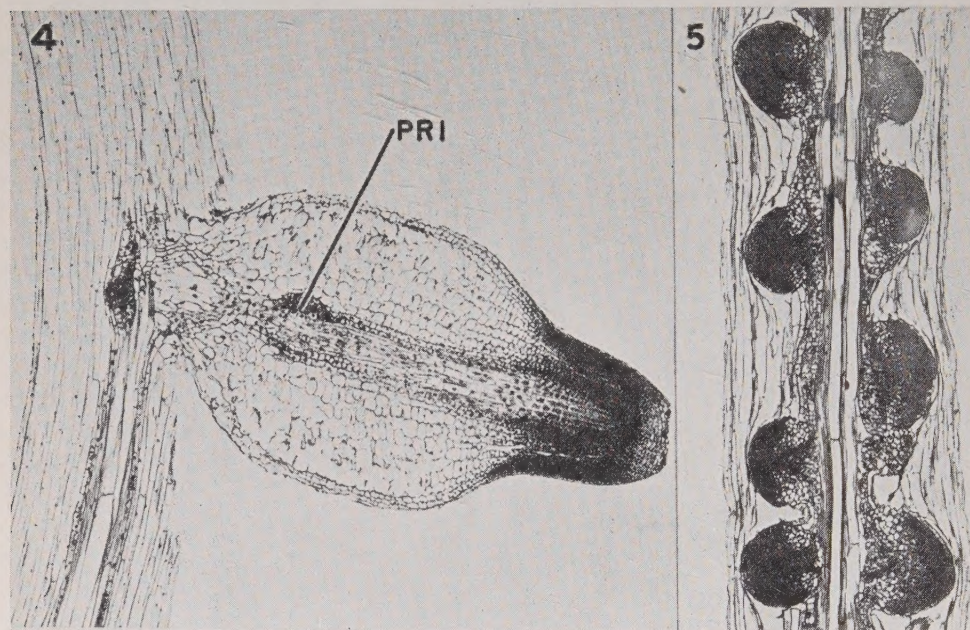
ridges of densely packed cells which often extend 200 μ or more along the stele. Whether root primordia are initiated is uncertain. No definite organization was seen in the ridges of meristematic cells, but it is possible that root primordia might eventually differentiate. Iso-

lated cells of the endodermis undergo tangential division within 96 hours after treatment, and within 144 hours many have divided.

In *Narcissus* roots no terminal swelling appears until 72 hours after treatment, and the maximum size is not reached for approximately 144 hours. Few or nu-

sion of the treatment, and at regular intervals up to 192 hours after the bulbs were replaced in nutrient solution.

Treatment does not result in tumor formation on roots of the John Ruskin variety. In fact, little variation from normal can be detected. Divisions in the cortex of the meristematic region appear



FIGS. 4, 5.—Longisections of mature regions of *Allium* roots 360 hours after treatment with naphthalene-acetic acid. Fig. 4, induced lateral root with young primordium developing. Fig. 5, section about 3 cm. above root apex with numerous root primordia.

merous root hairs develop on the tumor after 200 hours. Their maximum length never exceeds a small fraction of a millimeter.

Some division occurs in mature cells of the cortex, particularly in the early stages. No response was observed in either the pericycle or the endodermis.

INDOLE(3)ACETIC ACID.—Treatment with 0.001% for 24 hours was applied to John Ruskin *Tulipa* and *Narcissus* in the manner previously described. Roots were collected and fixed at the conclu-

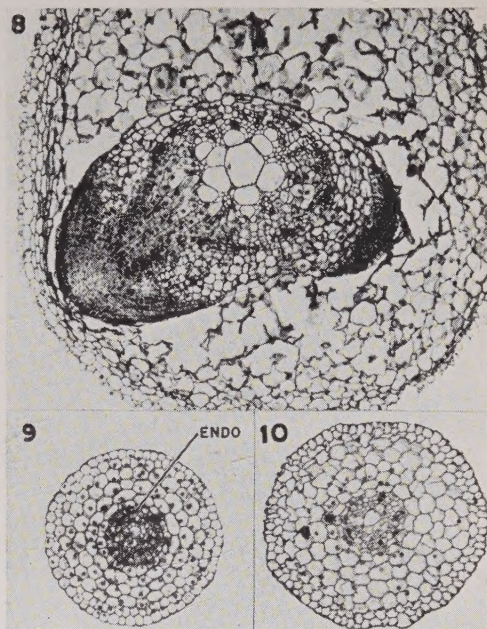
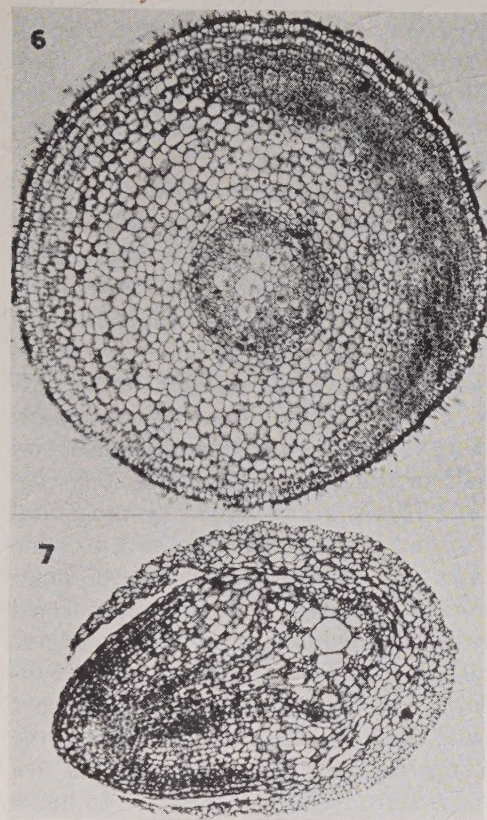
about 12 hours after treatment and eventually increase in number. These divisions at first are mostly radial, but later numerous tangential and diagonal ones occur. The daughter cells do not enlarge, and division in any cortical cell is completely independent of division in the adjacent cells.

Radial divisions in both pericycle and endodermis begin as early as 12 hours after treatment. Cells of the endodermis show tangential divisions 48 hours after treatment, and about 24 hours later

those of the pericycle also. At the same time, or perhaps somewhat earlier, the pericyclic cells undergo transverse division, resulting in rows of cubical cells

tions general proliferation does not occur, but localized regions of meristematic cells may indicate differentiation of root primordia.

Roots of *Narcissus* show no terminal enlargement at the conclusion of the



FIGS. 8-10.—Transection of roots treated with naphthaleneacetic acid: Fig. 8, *Allium*, 264 hours after treatment, showing developing primordia and torn cortex. Fig. 9, *Tulipa* Louis XIV, 120 hours, showing newly divided cells in endodermis and cortex and "apparent" binucleate condition in some cortical cells. Fig. 10, Louis XIV, 144 hours, showing effects of division in cortex and mitosis in mature cell. Endo, endodermis.

FIGS. 6, 7.—Transection of *Allium* root treated with naphthaleneacetic acid: Fig. 6, 120 hours after treatment, cut through base of tumor to show proliferation of cortex (cf. fig. 1) and early divisions in pericycle and endodermis. Fig. 7, 360 hours after treatment, showing emerging root primordium and development of vascular connections.

which are densely filled with cytoplasm and apparently meristematic. There are continued radial and tangential divisions in these tissues 120-192 hours after treatment, and the cortical cells adjacent to the endodermis also are affected. Proliferation is general, resulting in a compact band of four to six rows of cells around the stele. In more mature por-

treatment, but swelling is initiated within 24 hours and reaches its maximum in about 48 hours. Tumor formation results from enlargement of the cells of the cortex, with no indication of increase in the number of rows of cells in a longitudinal section. The treatment exerts a temporary stunting effect, stopping elongation for approximately 96 hours; but this is of short duration and then elongation is resumed. With renewal of elongation, the

tumor is left farther and farther behind the growing tip. Apparently, however, no further change occurs in its cells, for they remain almost isodiametric.

A few tangential and radial divisions occur in the cortical cells. The peripheral four or five rows undergo less enlarge-

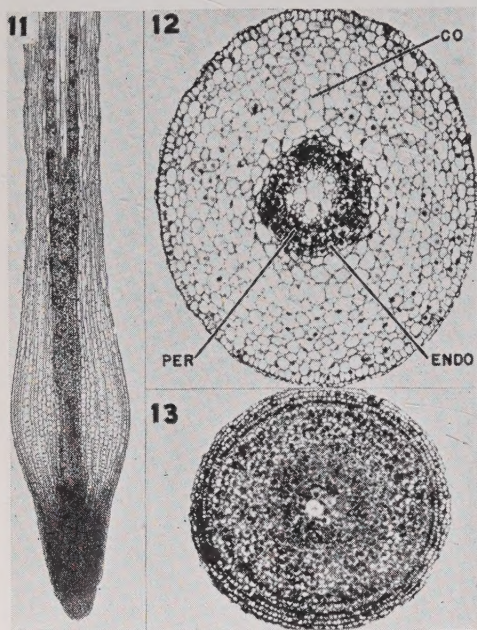
distance than is usual. In all instances division in mature cells occurs transversely.

INDOLE(3)BUTYRIC ACID.—Rooted bulbs of *Allium*, Louis XIV *Tulipa*, and *Narcissus* were treated with a 0.001% solution of this acid for 24 hours. Following treatment, roots were collected and fixed at 48-hour intervals up to and including 216 hours.

Swelling induced in *Allium* roots by this treatment reaches maximum size in approximately 24 hours, forming a spindle-shaped enlargement just above the root tip (fig. 11). First the pericyclic cells undergo repeated transverse divisions to form blocks of short meristematic cells. Tangential divisions follow shortly, forming a compact band several cells in thickness around the stele (fig. 12). This is accompanied by tangential divisions in the endodermis and, to some extent, by similar divisions of the inner cells of the cortex. The band thus formed varies in thickness from two to twelve cells and has an irregular external boundary. Development of this compact band of tissue is generally limited to the elongation and maturation regions. In the mature regions, within 72–216 hours after treatment, distinct areas of proliferation are formed. At first these are no more than compact groups of actively dividing cells, but later there are definite organization of tissues and differentiation of root primordia.

Mature cells of the cortex divide, but all divisions appear to be oriented normally in a transverse plane. Divisions in all planes occur in the immature cortical cells, the number progressively increasing. At 168 hours almost all the cells in the cortex of the meristematic region have undergone one or more divisions (fig. 13).

The only responses of roots of Louis



FIGS. 11–13.—*Allium* root: Fig. 11, 72 hours after treatment with indolebutyric acid, showing proliferation of pericycle and transition from normal tip to tumor. Fig. 12, transection through tumor, 72 hours after treatment with indolebutyric. Fig. 13, transection just below tumor, 168 hours after treatment, showing divisions of cortical cells in all planes. Co, cortex; Endo, endodermis; Per, pericycle.

ment than those nearer the center, and as a result the swollen region is bounded by a band of compact cells which remain active longer than most of the others.

Intercellular spaces, which occur even in the meristematic region of the root tip, are much larger in the swollen portion of the root. Mature cells are stimulated to divide in many instances, such divisions occurring as far as 16 mm. above the root tip, a considerably greater

XIV *Tulipa* to treatment consist of a few radial and tangential divisions in the young cortical cells and a few transverse divisions in the mature region.

Roots of *Narcissus* show no pronounced swellings 24 hours after treatment, but these are obvious within 72 hours. They are formed by the enlargement of individual cells rather than by increase in their number. There is a temporary stunting of root growth which lasts for approximately 168 hours, after which there is some slight renewal of elongation. Both radial and tangential divisions occur in the cells of the immature cortex but never abundantly. There are also a few transverse divisions in the mature cortical cells.

Transverse sections through the tumor show that the five or six peripheral rows of cortical cells are much more compactly arranged than are those nearer the stele. Intercellular spaces become numerous and large. Development of a limited number of stubby root hairs occurs on the lower portion of the tumor 120–216 hours after treatment.

β -NAPHTHOXYACETIC ACID.—Bulbs of both varieties of *Tulipa* and *Narcissus*, with abundant root development, were treated for 48 hours with a 0.002% solution of this acid. Roots were collected and fixed at 24-hour intervals from 48 to 144 hours.

Narcissus roots usually respond to treatment by tumor formation (figs. 14, 15), but there are exceptions where no swelling occurs. The roots are apparently permanently stunted. The tumors are formed by the enlargement of cortical cells, chiefly in a radial plane. Transition from the normal root tip may occur gradually or abruptly, involving only a few cell lengths. Roots collected and fixed at 72 and 120 hours after treatment have sparse development of root hairs on the

lower part of the tumor, and their maximum length is about 0.1 mm.

Transverse sections indicate that cell development and mitosis are normal during early phases (up to 96 hours), but in material fixed 120 hours or more after



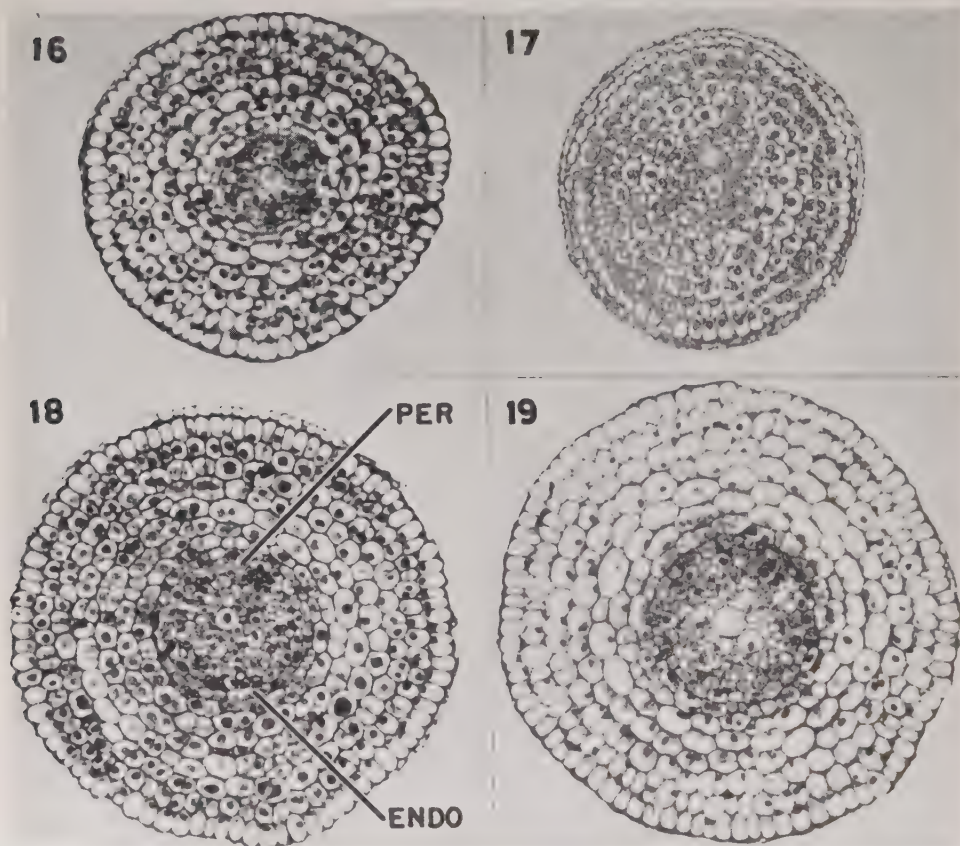
FIGS. 14, 15.—*Narcissus* root: Fig. 14, longitudinal section, 120 hours after treatment with naphthoxyacetic acid. Fig. 15, 144 hours after treatment with naphthyl acetamide.

treatment there are numerous divisions in all planes in the meristematic region. Mature cortical cells as far as 10 mm. from the root tip are stimulated to divide. Intercellular spaces occur in the swollen portion of the root. No changes were induced in the cells of the pericycle or in the stele of any root given this treatment.

Pronounced terminal swelling does not occur in roots of *Louis XIV*. Cortical

cells just back of the root tip undergo numerous divisions, but the daughter cells show little enlargement. The earliest divisions appear to be radial and occur mainly toward the periphery (figs. 16,

divide both radially and tangentially; the daughter cells thus formed remain densely filled with cytoplasm. These regions often appear to be associated with the protoxylem points, which might indi-



FIGS. 16-19.—Transsections of *Tulipa* Louis XIV roots treated with naphthoxyacetic acid: Fig. 16, 48 hours after treatment, showing numerous recently completed divisions in cortex. Fig. 17, 96 hours; similar divisions. Fig. 18, 120 hours; divisions in all directions in cortex and initiation of divisions in endodermis. Fig. 19, 144 hours; section cut at higher level, showing divisions in endodermis and pericycle. *Endo*, endodermis; *Per*, pericycle.

17), but of those occurring later, many are tangential (figs. 18, 19). Pericyclic cells are stimulated to divide transversely in certain regions, forming short rows of nearly isodiametric cells with large nuclei and dense cytoplasm. In older roots, killed 120 hours or more after treatment, both pericyclic and endodermal cells

cate early stages in the formation of root primordia; but development is so slight that no definite conclusions can be drawn.

Mature cells of the cortex are stimulated to divide as far as 10 mm. from the root tip and in a number of planes.

Roots of John Ruskin *Tulipa* respond

similarly but somewhat more slowly than those of Louis XIV. Pericyclic cells divide frequently, and the daughter cells are filled with dense cytoplasm; but despite their meristematic appearance, no proliferation appears to take place.

α -NAPHTHYL ACETAMIDE.—Rooted bulbs of *Allium*, Louis XIV *Tulipa*, and *Narcissus* were treated for 48 hours with a 0.001% solution. Roots were collected and killed at 48-hour intervals up to 192 hours.

Allium roots show considerable swelling, forming bulbous tumors. Lateral root primordia develop rather quickly, and mitosis occurs in cells throughout the terminal 10 mm. of the roots, both in the stele and in the cortex. The tumors formed on the roots are similar to those previously described and result from cell enlargement rather than from increase in cell number. Transition from the normal root tip takes place within 10–12 cell lengths. Few of the enlarged cells of the cortex show either recently completed divisions or mitotic figures, indicating little meristematic activity.

Root primordia are initiated within 48 hours following treatment, but the number at this time is few; at 144 hours after treatment their number has increased considerably. The position of the primordia appears to have no effect on their size or rate of development, since those nearest the root tip are not necessarily the youngest or the smallest. Within 192 hours after treatment, some primordia have pushed halfway through the cortex. The steps in the development of primordia are essentially the same as those previously discussed, but none developed sufficiently to emerge from the main root.

Some enlargement of the hypodermal cells (fig. 20) occurred in *Allium*. The enlargement, however, is general and not

limited to regions over the protoxylem points, as was described by BURSTRÖM (7) for wheat.

Louis XIV *Tulipa* shows relatively little response to treatment. A very slight swelling of the root tip occurs,



FIGS. 20, 21.—Fig. 20, *Allium* root, 48 hours after treatment with naphthyl acetamide, showing some enlargement of hypodermal cells. Fig. 21, *Narcissus*, 96 hours, showing disruption of cortex and compact layer of cells around periphery.

caused by limited enlargement of the cortical cells, which in the meristematic region show numerous divisions, at first radial and later both tangential and diagonal. The resulting daughter cells do not enlarge or show further development.

Cells of the pericycle undergo transverse division, and the daughter cells contain dense cytoplasm and have large nuclei, giving them the appearance of meristematic cells; but no proliferation

occurs and no root primordia are initiated. Mature cortical cells are stimulated to divide, and a number were observed in various stages of mitosis.

Within 48 hours after treatment, roots of *Narcissus* show prominent swellings a short distance above the tips, although pronounced enlargement does not always occur (fig. 15). The tumors result from cell enlargement only, involving chiefly the inner cortical cells—the peripheral cells remaining normal size. Continued enlargement of the cortical cells results in the formation of large schizogenous air spaces (fig. 21). Pericyclic cells show none of the activity shown by *Allium* and *Tulipa*, and no root primordia are initiated.

TRYPTOPHANE.—Roots were exposed to a 0.002% solution for 48 hours, after which the bulbs were returned to a balanced nutrient solution. Roots were killed at regular intervals from 24 to 192 hours after treatment.

Roots of *Allium* show little morphological change after treatment with tryptophane. No terminal swelling occurs, and there is only slight effect on cell polarity, since almost all mitotic figures are oriented normally. Up to 48 hours few of the young cortical cells show signs of recently completed division, although such divisions become more frequent later.

The endodermal cells divide tangentially, beginning about 72 hours after treatment, but become numerous only after 96 hours. The pericyclic cells, however, do not undergo the usual transverse divisions reported for the preceding substances. Pericyclic and endodermal cells gradually become less densely filled with cytoplasm as they mature. Neither initiation of root primordia nor proliferation of the pericycle takes place.

Mitosis occurs only in the meristemat-

ic region, the terminal 1.5–2 mm. of the root. In none of the material observed were the mature cells of the cortex stimulated to divide. Maturation of the tissues, particularly those of the stele, seems to occur more slowly in roots treated with tryptophane than in control material.

Roots of both tulips respond to tryptophane much as do those of *Allium*. Perhaps division in the endodermal and cortical cells occurs somewhat earlier in the John Ruskin. Approximately 72 hours after treatment, however, certain regions of the pericycle undergo transverse cell division, forming rows of short, almost isodiametric cells with dense cytoplasm. Tangential divisions take place in these cells but never become abundant. These responses occur at some distance proximal to the root tip.

The pericycle continues development from 96 to 192 hours after treatment, but the development is limited to definite regions. At first there is a mass of meristematic cells which shows no definite organization, but later there is a certain amount of organization. In a few instances the development of the proliferation is inward, resulting in some displacement of the vascular elements. Some cells of the proliferation take on the structure and appearance of wound tracheids, and it is possible that this leads to the formation of vascular tissue within the proliferation and the formation of vascular connections with the xylem of the root.

Narcissus roots develop slight spindle-shaped swellings near the tips, increasing the root diameter by about one-half. Cell polarity in the meristematic region is unaffected by treatment, and practically all the divisions are transverse. Pericyclic cells lose their dense cytoplasm in the elongation and maturation regions, and few radial and tangential divisions

occur. Mitosis is not promoted in the cells of mature tissues.

Discussion

The responses of these roots to treatment with the various growth-regulating substances extend, and to some extent agree with, the histological observations of LEVAN (17) and the general observations of BORGSTROM (6), GAVAUDAN (10), LANE (16), and others. Formation of terminal tumors on treated roots confirms the discussion and the illustrations presented by BORGSTROM. However, some of the data are not in agreement with the results of LEVAN and BURSTRÖM. The latter states that the rate of mitosis (in roots of wheat) is unchanged by the addition of heteroauxin (indole(3) acetic acid), but that such treatment does exert an influence on the direction of cell divisions, some being changed from transverse to radial or tangential; and thus an increase in the number of rows of cells is brought about, giving an increased root diameter. LEVAN states that "it will be seen from the longitudinal section that a larger number of cell rows is present than normally. This is due to the fact that the meristematic cortical cells under the influence of the growth substances start numerous mitoses. These mitoses are oriented in the transverse axis of the root, tangentially or radially." He then states that such divisions are not a necessary condition for the subsequent swelling of the cells, and that frequently the divisions are lacking.

In the present investigation there was no indication of increase in the number of vertical rows of cortical cells in the transition from the normal to the enlarged regions of the roots (figs. 2, 3, 11, 14, and 15), and few cases of cell division in any plane were observed in the cortical

cells of the tumors. Divisions in many planes frequently were seen in the immature cortex below the tumor, however, and mitosis in mature cortical cells above the tumor was not uncommon. It is evident that treatment does affect cell polarity, for irregular divisions in the root tips did not occur in control material.

BURSTRÖM also described a hypertrophy of the hypodermal cells over the protoxylem strands in the mature region of roots of wheat seedlings, the enlargement becoming so extensive as to rupture the epidermis. Some hypertrophy of the hypodermis may occur in roots of *Narcissus* following various treatments, but it is always general and never very pronounced; and its occurrence is erratic. A similar condition occurred in the roots of *Allium* (fig. 20).

KRAUS and co-workers (14) have reported that in stems of Red Kidney bean treated with 3% indoleacetic acid in lanolin there is acceleration of mitoses, so that many cells become multinucleate. This condition was not found in root tumors of any of the plants investigated. In many instances, and in all roots studied, there were cells which appeared to be binucleate; but more critical observation showed that the nuclei were on entirely different focal levels, and almost invariably a film of cytoplasm and a cell wall lay between them. Longitudinal sections of roots frequently showed the nucleus of one cell resting against the lower wall, while the nucleus of the cell below lay against the upper wall. If observed in transverse section it might easily be interpreted as a cell containing two nuclei.

The ideas of the relationship between nuclear size and ploidy advanced by BERGER (3) cannot be taken too literally in studying an entire root section, although it probably is true that within the cells of any specific tissue such a rela-

tionship exists. For example, in a transverse section of a root the nuclei of the cortical cells are fairly large and prominent, but nuclei of the cells of the stele may be only one-fourth or one-eighth as large. If the same material is examined in longitudinal section, the nuclei of the cortical cells will be spherical or nearly so, while those in the stele are so elongated in some cases as to appear vermiform. Nuclear volume, however, may be essentially the same.

MARMER (19) has reported that the main or "primary" roots of wheat seedlings treated with indoleacetic, indolebutyric, or indolepropionic acid showed thickening, flattening, curling, and excessive root-hair formation at pH 4.6. In my investigations, root-hair formation was observed only after 120 hours or more in nutrient solution subsequent to treatment. The pH of the nutrient solution was approximately 5.5 at the time the bulbs were placed in it after treatment; and while no attempt was made to confirm this fact, it is probable that the physiological activity of the immersed roots resulted in the shifting of the pH to a point where root-hair formation was encouraged. An investigation of this phase of the work, using controlled pH, might be of interest.

That mitosis appears to be normal in those regions of *Lilium* stems showing response to treatment with indoleacetic has been reported by BEAL (1). He found the diploid chromosome number to be present without exception. This is in agreement with these observations of roots of *Allium*, *Tulipa*, and *Narcissus* treated with indoleacetic acid and other growth substances. In the root primordia formed in *Allium* roots many mitotic figures were observed; and as far as could be determined, all nuclei had the diploid number of chromosomes.

LEVAN found that normal division occurred in most meristematic cells of *Allium*, but that some developments in the enlarged cortical tissues were of particular interest, among these being the occurrence of mitosis as far as 3 mm. from the root apex. LEVAN also found that while cells at the apex showed a normal diploid chromosome number, the majority of those farther back showed tetraploidy, and many of those still farther back showed octoploidy. In *Tulipa*, *Allium*, and *Narcissus*, mitosis occurred as far as 10-16 mm. from the root apex, far beyond the range of the terminal tumor; but no increase in chromosome number was observed in either *Tulipa* or *Narcissus*. Cells with $3n$ and $4n$ chromosomes were observed in *Allium*, but the number was sufficiently small to lie within the range of the normal occurrence of polyploid cells. As stated before, mitosis in the enlarged cortical cells was exceedingly rare.

Summary

1. Bulbs of *Allium cepa*, *Tulipa* (vars. John Ruskin and Louis XIV), and *Narcissus* (var. Paper White) were rooted and treated with dilute solutions of various growth-regulating substances.

2. Roots of *Allium* and *Narcissus* responded quickly to treatment with all substances except tryptophane by the formation of more-or-less globular terminal tumors. Tumors induced by tryptophane generally were longer, more slender, and somewhat more spindle-shaped. Roots of *Tulipa* showed little enlargement. Root-tip enlargement, where present, was due to an increase in the size of the cortical cells and not to an increase in the number of their rows.

3. Treatment resulted in the slowing down or complete cessation of root elongation. In some cases the effect was only

temporary, but in others it was permanent.

4. Histological responses occurred first in the meristematic region, chiefly in the cortex, where cells underwent division in all planes.

5. Division in the enlarged cortical cells was rare; but mature cortical cells above the tumors and as far as 16 mm. above the root apex were stimulated to divide. Such divisions generally were typical and in a transverse plane.

6. Pericyclic cells first responded by transverse divisions, forming rows of isodiametric cells with dense cytoplasm and large nuclei. These cells later divided radially and tangentially, resulting in considerable proliferation.

7. Proliferation did not occur in roots of *Narcissus*. Radial and tangential divi-

sions occurred in the endodermal cells, but no true proliferation was apparent.

8. Proliferation of pericycle and development of numerous root primordia occurred in roots of *Allium*. Proliferations also occurred in both varieties of *Tulipa*, but the formation of definite, organized root primordia was not observed.

9. A few polyploid cells were seen in *Allium* roots, but none was found in either *Tulipa* or *Narcissus*.

10. Binucleate or multinucleate cells were not observed in any root subjected to any treatment.

The writer acknowledges with sincere thanks the advice and aid given by Dr. J. M. BEAL of the University of Chicago during the course of this investigation.

FLORIDA DEHYDRATION COMPANY
ZELLWOOD, FLORIDA

LITERATURE CITED

1. BEAL, J. M., Histological responses of three species of *Lilium* to indoleacetic acid. *BOT. GAZ.* **99**:881-911. 1938.
2. ———, Effects of indoleacetic acid on thin sections and detached segments of the second internode of the bean. *BOT. GAZ.* **102**:366-372. 1940.
3. BERGER, C. A., Some criteria for judging the degree of polyploidy of cells in the resting stage. *Amer. Nat.* **75**:93-96. 1941.
4. BORTHWICK, H. A., HAMNER, K. C., and PARKER, M. W., Histological and microchemical studies of the reactions of tomato plants to indoleacetic acid. *BOT. GAZ.* **98**:491-519. 1937.
5. BONNER, JAMES, and KOEPFLI, J. B., Inhibition of root growth by auxins. *Amer. Jour. Bot.* **26**:557-565. 1939.
6. BORGSTROM, GEORG, Influence of growth-promoting chemicals on roots of *Allium*. *Botan. Notiser* **1939**:207-220. 1939.
7. BURSTRÖM, HANS, Influence of heteroauxin on cell growth. *Ann. Agr. Col. Sweden* **10**. 1942.
8. COOPER, W. C., Hormones and root formation. *BOT. GAZ.* **99**:599-614. 1938.
9. DERMEN, HAIG, Intracellular polyploidy in bean induced by naphthaleneacetic acid. *Jour. Hered.* **32**:133-138. 1941.
10. GAVAUDAN, P., and GAVAUDAN, N., Tumefaction des racines par les substances modificatrices de la carcyonine. *Compt. Rend. Séances Soc. Biol.* **131** (16):168-171. 1939.
11. HAMNER, K. C., Histological responses of *Mirabilis jalapa* to indoleacetic acid. *BOT. GAZ.* **99**:912-954. 1938.
12. HARRISON, B. F., Histological responses of *Iresine lindenii* to indoleacetic acid. *BOT. GAZ.* **99**:301-338. 1938.
13. KRAUS, E. J., Histological responses of bean plants to l-tryptophane. *BOT. GAZ.* **102**:602-622. 1941.
14. KRAUS, E. J., BROWN, NELLIE A., and HAMNER, K. C., Histological reactions of bean plants to indoleacetic acid. *BOT. GAZ.* **98**:370-420. 1936.
15. KRAUS, E. J., and MITCHELL, J. W., Histological and physiological responses of bean plants to alpha naphthalene acetamide. *BOT. GAZ.* **101**:204-225. 1939.
16. LANE, R. H., Inhibition of roots by growth hormones. *Amer. Jour. Bot.* **23**:532-534. 1936.
17. LEVAN, ALBERT, Cytological phenomena connected with the root swelling caused by growth substances. *Hereditas* **25**:87-96. 1939.
18. MACHT, D. I., and GRUMBEIN, MARY L., Influence of indoleacetic, indolebutyric, and naphthaleneacetic acids on roots of *Lupinus albus* seedlings. *Amer. Jour. Bot.* **24**:457-460. 1937.
19. MARMER, DINA R., Growth of wheat seedlings in solutions containing chemical growth substances. *Amer. Jour. Bot.* **24**:139-145. 1937.
20. THIMANN, K. V., Auxins and the growth of roots. *Amer. Jour. Bot.* **23**:561-569. 1936.
21. ZIMMERMAN, P. W., and HITCHCOCK, A. E., Response of roots to "root-forming" substances. *Contr. Boyce Thompson Inst.* **7**:439-445. 1935.

